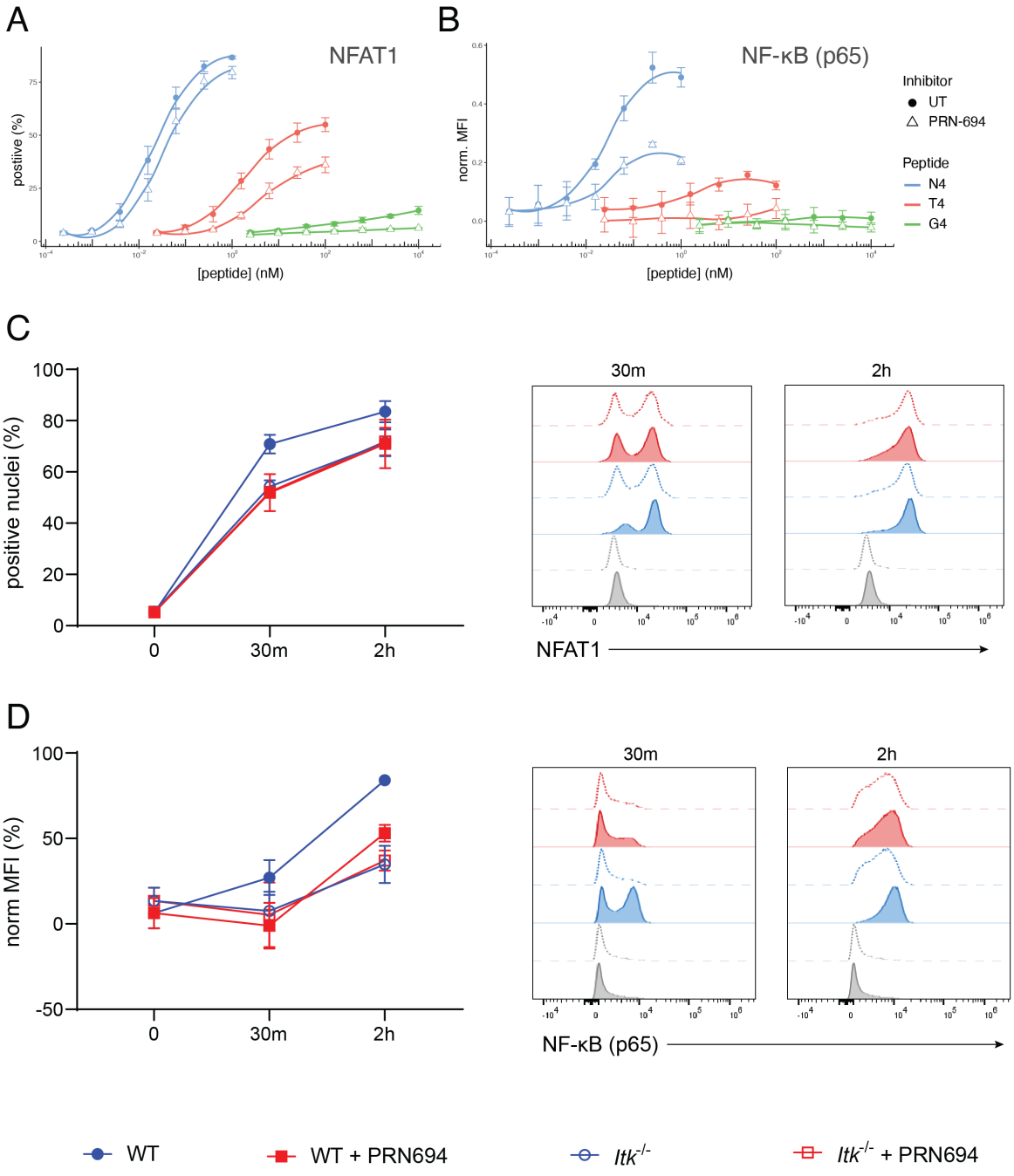
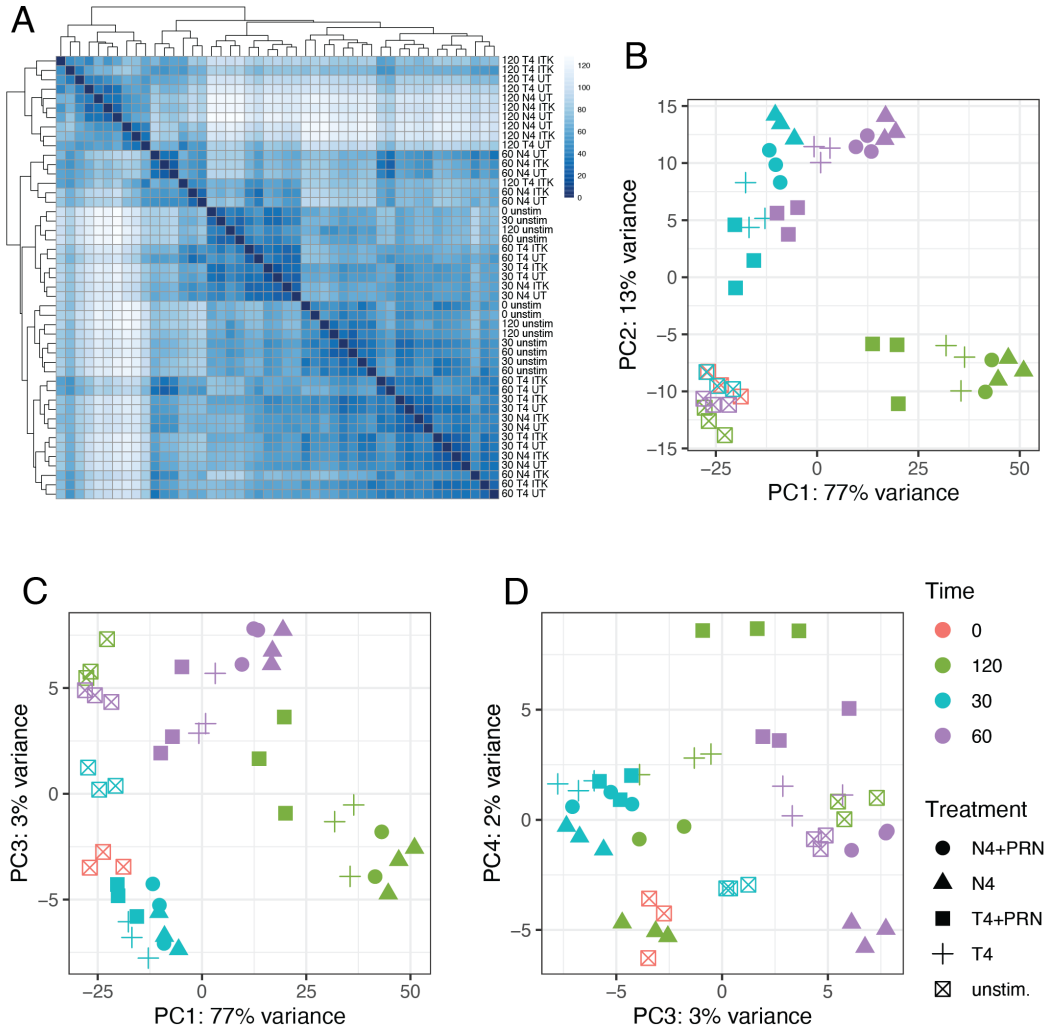


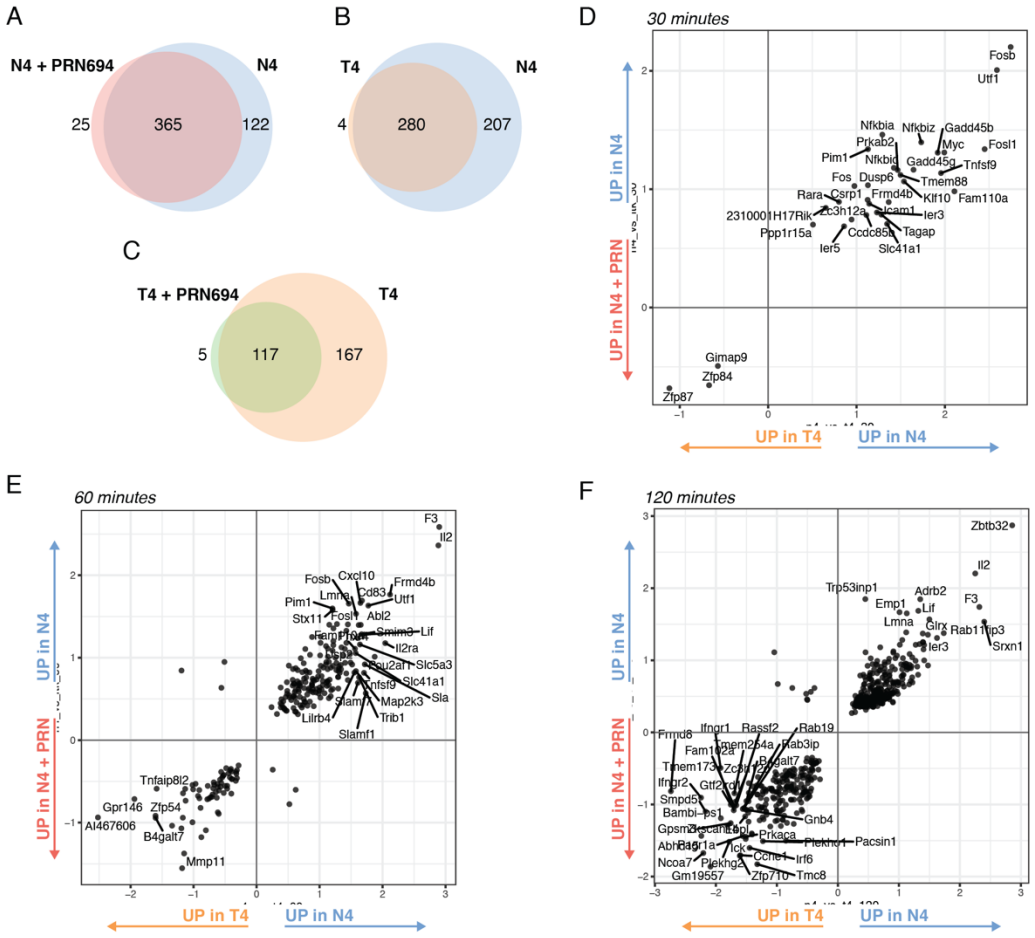
Supplemental Figure 1. PRN694 differentially affects NF-κB and NFAT1 responses during variable peptide stimulation.

(**A-B**) Line plots depicting NFAT1 or NF-κB (p65) activation in OT-I nuclei in response to 30-minute stimulation by WT splenocytes loaded with indicated doses of N4, T4, or G4 peptide, with or without treatment with 50 nM PRN694. Error bars indicate s.e.m. (**C-D**) Line plots and histograms depicting NFAT1 or NF-κB (p65) activation in response to 30-minute or 2 hour stimulation of OT-I WT or *Itk*$^{-/-}$ cells by WT splenocytes loaded with 100 nM N4 peptide, with or without treatment with 50 nM PRN694. Line plots represent data compiled from three independent experiments and show mean+/-SD.



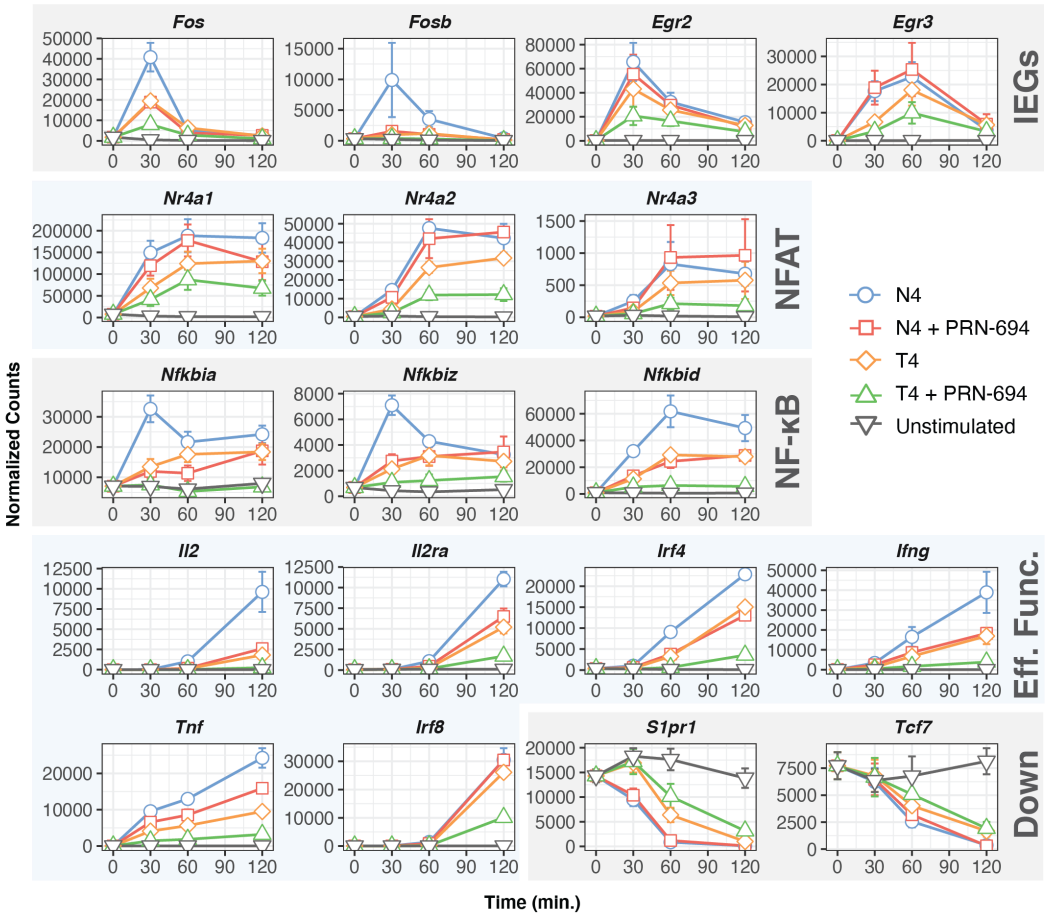
Supplemental Figure 2. RNA-seq replicate correlation and principal component analysis.

(**A**) Sample-to-sample correlation to compare RNA-seq replicates. (**B-D**) Principal component analysis of all RNA-seq replicates across all stimulation conditions.

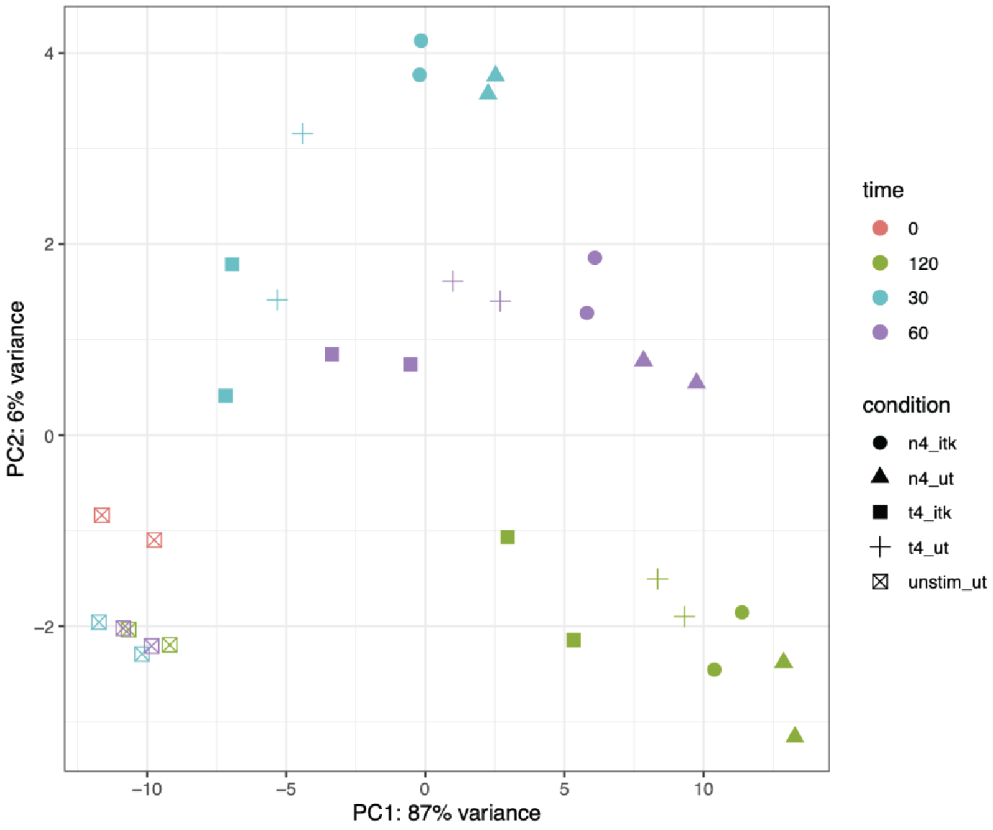


Supplemental Figure 3. Graded TCR signaling induces a single transcriptional program.

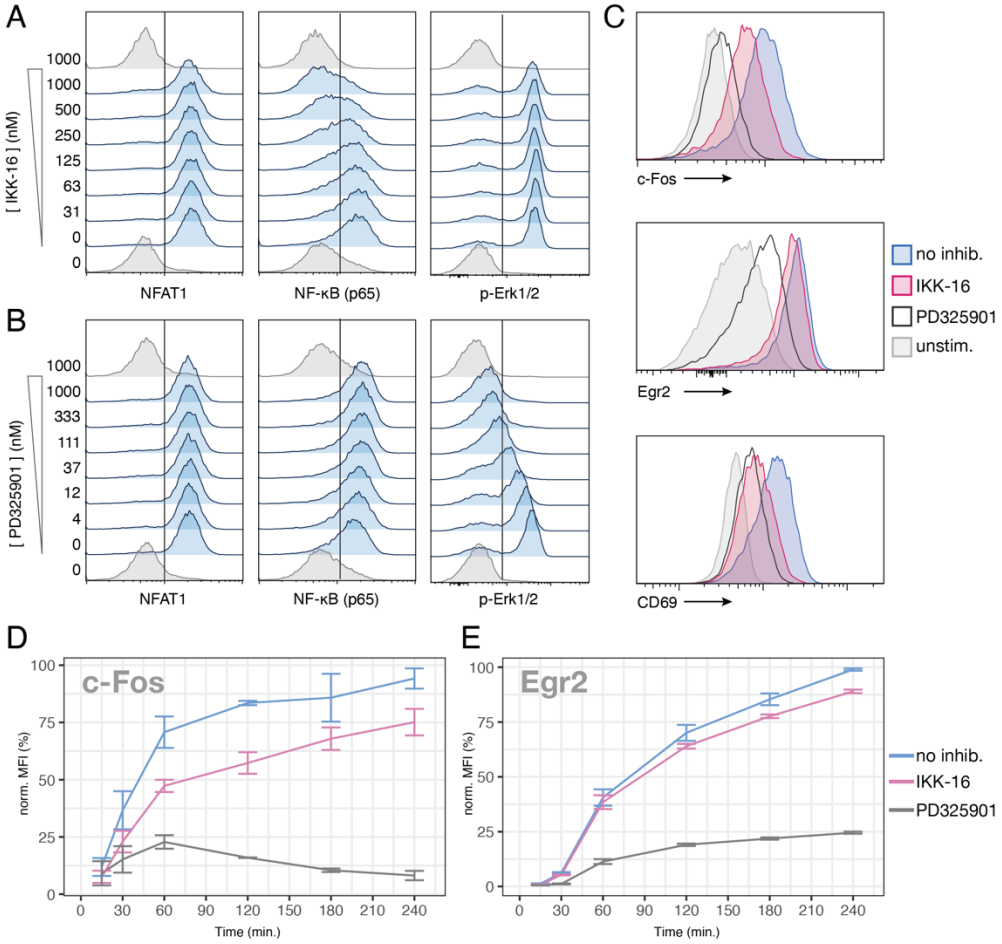
(**A-C**) Indicated overlap of induced genes identified in each tested TCR signaling condition. N4 = blue (*n*=487), T4 = orange (*n*=284), N4 + PRN694 = red (*n*=390), T4 + PRN694 = green (*n*=122). (**D-E**) The effect of stimulation with weaker affinity peptide vs treatment with PRN-964. Linear correlation of Log2 fold-change values determined by differential expression analysis of either N4/N4+PRN694 or N4/T4 comparisons at each time point.



Supplemental Figure 4. Example patterns of detected early transcripts.
Line plots depicting normalized count data of selected gene transcripts over the course of the 2-hour RNA-seq experiment. Colors are consistent with other figures. Data represent three separate biological replicates. All genes presented are significantly differentially expressed compared to control at least at one time point. Error bars represent s.e.m.



Supplemental Figure 5. Principal component analysis of ATAC-seq replicates. Principal component analysis of batch-corrected peak coverage data for all ATAC-seq replicates across all stimulation conditions.



Supplemental Figure 6. Specific NF-κB inhibition differentially reduces c-Fos protein accumulation.

(**A-B**) Histograms depicting effect of titration of IKK-16 (A) or PD325901 (B) on NFAT1, NF-κB, and p-Erk1/2 activation after 1-hour simulation with N4 peptide. (**C**) Histograms demonstrating the effect of 500 nM IKK-16 or 1000 nM PD325901 on c-Fos, Egr2, and CD69 protein expression in OT-I cells stimulated with N4 peptide for 2 hours. (**D-E**) Line plots of depicting 500 nM IKK or 1000 nM PD325901 treatment on c-Fos (D) and Egr2 (E) protein accumulation over 4 hours stimulation with N4 peptide. Compiled are three separate experiments, error bars indicate s.e.m.

Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
HALLMARK_TNFA_SIGNALING_VIA_NFKB	33/369	141/4143	1.16E-07	4.67E-05	4.02E-05	*Rel/Gadd45b/Atp2b1/Dusp2/Sat1/Plaur/Myc/Nfe2l2/Cdkn1a/Kdm6b/Traf1/Map3k8/Tgif1/Nfat5/Fosb/Ier3/Il15ra/Maff/Fosl1/Tank/Irf1/Ppp1r15a/Sgk1/B4galt5/Klf4/Nfkbia/Tnfaip8/Cxcl10/Icam1/Zbtb10/Socs3/Birc2/Lif*	33
HALLMARK_IL2_STAT5_SIGNALING	25/369	138/4143	0.000389162	0.070278633	0.060577116	*Gadd45b/Slc1a5/Spry4/Odc1/Myc/Traf1/Nfkbiz/Map3k8/Igf2r/Bcl2l1/Ccnd2/Ncs1/Maff/Uck2/Gbp3/Ndrg1/Rnh1/Batf/Cst7/Ctsz/Cxcl10/Itga6/Dcps/Lif/Ahr*	25
WP_ASSOCIATION_BETWEEN_PHYSICOCHEMICAL_FEATURES_AND_TOXICITY_ASSOCIATED_PATHWAYS	10/369	35/4143	0.000669137	0.070278633	0.060577116	*Pfn1/Rock2/Myc/Cdkn1a/Ppp2ca/Cbl/Mapk1/Mapk8/Map2k4/Ctnnb1*	10
WP_TNF_ALPHA_SIGNALING_PATHWAY	17/369	82/4143	0.000697555	0.070278633	0.060577116	*Rel/Tbk1/Ppp2ca/Traf1/Map3k8/Apaf1/Bcl2l1/Tank/Nfkbia/Cdc37/Mapk1/Mapk8/Birc2/Map2k4/Kras/Rac1/Nfkbib*	17
WP_NOTCH_SIGNALING_PATHWAY_NETPATH	10/369	38/4143	0.001351915	0.081061021	0.069871064	*Myc/Hdac1/Adam17/Cdkn1a/Rbpj/Jak2/Numb/Stat3/Hif1a/Maml3*	10
WP_PROLACTIN_SIGNALING_PATHWAY	13/369	58/4143	0.001385457	0.081061021	0.069871064	*Myc/Cbl/Ctsd/Irf1/Nfkbia/Mapk1/Jak2/Mapk8/Itgb1/Stat3/Socs3/Rac1/Nfkbib*	13
HALLMARK_INFLAMMATORY_RESPONSE	18/369	95/4143	0.001486907	0.081061021	0.069871064	*Slc7a1/Atp2b1/Emp3/Lta/Plaur/Myc/Cdkn1a/Atp2a2/Il15ra/Irf1/Nfkbia/Hif1a/Cxcl10/Icam1/Slc11a2/Il1r1/Lif/Ahr*	18
WP_SMALL_CELL_LUNG_CANCER	12/369	52/4143	0.001609152	0.081061021	0.069871064	*Gadd45b/Myc/Cdkn1a/Traf1/Apaf1/Traf6/Bcl2l1/Nfkbia/Itgb1/Itga6/Birc2/Nfkbib*	12
WP_STRUCTURAL_PATHWAY_OF_INTERLEUKIN_1_IL1	11/369	46/4143	0.001841229	0.082446139	0.071064976	*Myc/Tollip/Map3k8/Traf6/Tank/Nfkbia/Mapk1/Mapk8/Il1r1/Map2k4/Nfkbib*	11
WP_IL6_SIGNALING_PATHWAY	9/369	34/4143	0.002240158	0.084856854	0.073142907	*Hdac1/Bcl2l1/Irf1/Mapk1/Jak2/Stat3/Socs3/Map2k4/Rac1*	9
WP_ARYL_HYDROCARBON_RECEPTOR_NETPATH	8/369	28/4143	0.002316192	0.084856854	0.073142907	*Myc/Nfe2l2/Cdkn1a/Ccl1/Cdc37/Mapk1/Kras/Ahr*	8

Supplemental Table 1. Gene ontology results of unique genes within ATAC-seq Cluster 1.

Enrichment statistics of Wikipathways (WP_) and MSigDB Hallmark (H_) terms reported for gene annotations near peaks unique to Cluster 1 (gene names found in multiple clusters were removed before analysis).